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CHANGES IN THE ORGAN OF HEARING PRODUCED BY SOUND.(U)

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CHANGES IN THE ORGAN OF HEARING PRODUCED BY SOUND

David J. Lim
William Melnick

JULY 1982

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Air Force Aerospace Medical Research Laboratory
Aerospace Medical Division
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TECHNICAL REVIEW AND APPROVAL

AFAMRL-TR-80-127

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Henning E. von Gierke

HENNING E. VON GIERKE, Dr Ing
Director
Biodynamics and Bioengineering Division
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This report summarizes the research done for the Air Force from March 1, 1977, to May 1, 1980, by the Otological Research Laboratories at the Ohio State University College of Medicine, David J. Lim, M.D. and William Melnick, Ph.D., principal investigators. The research was funded by Air Force contract F33615-77-C-0512. The data have been presented at national and international meetings concerning auditory function and ototrauma; in addition, twelve articles have been published or are being submitted for publication.

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FINAL REPORT

"CHANGES IN THE ORGAN OF HEARING PRODUCED BY SOUND"

This report summarizes the research done for the Air Force from March 1, 1977, to May 1, 1980, by the Otological Research Laboratories at the Ohio State University College of Medicine, David J. Lim, M.D. and William Melnick, Ph.D., principal investigators. The research was funded by Air Force contract F33615-77-C-0512. The data have been presented at national and international meetings concerning auditory function and ototrauma; in addition, twelve articles have been published or are being submitted for publication.

A. <u>**Normal Anatomy** of Auditory Periphery (Basic Studies)</u>

1. <u>Fine Morphology of the Tectorial Membrane</u>

Recent laboratory data have pointed to the importance of understanding the way in which the sensory cilia - tectorial membrane coupling contributes to auditory sensory transduction. The nature of this coupling has become a focal point of debate. In order to elucidate the relationship between the inner and outer hair cell ciliary coupling mechanisms, fresh organs of Corti, preserved in either artificial endolymph or artificial perilymph, were studied using the inverted phase contrast microscope and cinephotography. The results show that the tectorial membrane has all the known substructural details in the fresh state. Although free-moving cilia of the inner hair cells were not observed, outer hair cell ciliary attachment was strongly indicated in this study. The difficulty of showing free-moving inner hair cell cilia, if they are not firmly attached to the tectorial membrane, is probably due to the stiffness of the cilia in the fresh condition, unlike in the fixed state in which they are flaccid.

For the developmental aspect of the tectorial membrane and sensory cilia, kittens were studied. The results show that the tectorial membrane develops in two stages. The major portion develops from the tall columnar cells, which are the equivalent of the interdental cells, and as the organ of Corti matures the tectorial membrane begins to cover the sensory cilia. The primary tectorial membrane basically covers the greater epithelial ridge, and the tectorial membrane covers the lesser epithelial ridge. A major portion of the tectorial membrane appears to be formed by the tectorial membrane produced by the supporting cells of the organ of Corti, namely the cells equivalent to the phalangeal cells. The lip portion of the tectorial membrane is firmly attached to the third row of Deiters' cells, forming a complete seal along the organ of Corti. But as the organ of Corti matures this seal is broken. These findings are consistent with earlier observations made on the mature organ of Corti that the subtectorial space is open to endolymph and that the inner and outer hair cell ciliary couplings might be different. The outer hair cell sensory cilia are firmly coupled to the tectorial membrane, but the inner hair cells are not.

For a detailed report see Addendum I.

2. <u>Cochlear Anatomy Related to Cochlear Micromechanics</u>

The sensory cilia of the mammalian cochlea show an orderly gradation in height along the length of the cochlea. The cilia are taller in the apex and shorter in the base. Differences in ciliary gradation also exist between the different rows of outer hair cell cilia along the length of the cochlea. There are gradations in width and thickness in the moveable portion of the tectorial membrane paralleling those in the basilar membrane. There are also gradations in the relationship between the tectorial membrane and the slope of the reticular lamina along the length of the cochlea. This suggests that there may be additional mechanical fine-tuning capability built into the organ of Corti besides the basilar membrane. The tectorial membrane is firmly attached to the outer hair cell cilia along the entire length of the cochlea in all species examined. The inner hair cell cilia do not have the same firm attachment to the tectorial membrane as outer hair cell cilia. This suggests that the modes of mechanical coupling between the tectorial membrane and the inner and outer hair cell cilia are different.

For a detailed report see Addendum II.

3. <u>Ultra-anatomy of Sensory End Organs in the Labyrinth and Their Functional Implications</u>

This study compared the micromechanics of the auditory system and of the vestibular system. It is postulated that the outer hair cell cilia are firmly embedded in the tectorial membrane, whereas the inner hair cell cilia are freestanding. This arrangement would allow the outer hair cells to be most effectively stimulated by a shearing motion of the tectorial membrane and the inner hair cell cilia by drag (or motion) of the endolymph caused by the shearing motion of the tectorial membrane and reticular lamina of the organ of Corti. A similar anatomic relationship exists in the vestibular sensory organs. In the vestibular gravity receptor organs the otoconial membrane has specialized areas: the striola, or central region, which is perforated; and the peripheral area, which is thick. Many of the sensory cilia in the peripheral area are tall and made up of type 1 sensory cells, and the remainder of the cells are type 2 and their cilia are much shorter. In the central region (striola) the sensory cilia, regardless of type of sensory cell, are extremely short. This system is analogous to the auditory system, in which the sensory cilia of the inner hair cells are freestanding and the sensory cilia of the outer hair cells are embedded in the tectorial membrane. Therefore, the suggestion has been made that there is a dichotomy in sensory transduction throughout the mammalian inner ear, both auditory and vestibular.

For a detailed report see Addendum III.

4. Current Review of SEM Techniques for Inner Ear Sensory Organs

Special SEM applications, such as fresh tissue examination, fresh frozen freeze-fractured and freeze-dried tissue examination, and x-ray analysis, in inner ear study were examined with emphasis on their merits and demerits.

Using Lane's environmental control stage, a wet piece of cochlea was examined. Problems encountered were charging of the specimens, rapid evaporation, and ice formation. Even with these problems, a usable image of the basilar membrane was obtainable.

Freeze-dried or freeze-fractured fresh inner ear tissues provided well-preserved cupulae, organic substances of the endolymph and organ of Corti, and, to a lesser extent, tectorial membranes. One of the major problems with the freeze-drying technique was ice crystal damage. X-ray microanalysis of the freeze-dried endolymph and tectorial membrane revealed that it appears not to be saturated with endolymph.

Using the tectorial membrane as an example, the reliability of SEM findings was studied by comparing fresh and fixed tissue with the light microscope, transmission electron microscope (TEM) and SEM. Substructures (cover net, marginal band, Hensen's stripe, and fibrotic texture of the membrane proper) observed by SEM in fixed tectorial membranes were also clearly seen in fresh tissue examined using a phase contrast microscope, supporting the credibility of SEM findings. Trabeculae of Hensen's stripe, which were observed using the SEM on fixed tissue, were also demonstrated by TEM, as well as SEM using freeze-fractured fresh organs of Corti, giving strong supportive evidence that the SEM findings are reliable if a high standard of specimen preparation is met.

For a detailed report see Addendum IV.

B. Acoustic Trauma Studies

1. Trauma of the Ear from Infrasound

Twenty-seven chinchillas received continuous or intermittent infrasound (1, 10, 20 Hz) at 150, 160, or 170 dB SPL; there were five controls. Serial sections of the temporal bones were examined using light microscopy. Pathologies noted were tympanic membrane perforation, stapes subluxation, bleeding from the middle ear mucosa and tensor tympani, strial pathology, Reissner's membrane rupture, endolymphatic hydrops, saccular wall rupture, hair cell damage, and blood in the cochlear scalae. Continuous infrasound was more damaging than intermittent. Only continuous infrasound produced saccular pathology and perforations of the tympanic membrane. Of the other pathologies observed, continuous infrasound exposures produced 67% of the hair cell damage, 73% of the bleeding in the cochlear scalae, 83% of the strial pathology, and 78% of the cases of cochlear hydrops. 170-dB infrasound exposures produced the highest percentage of ears with pathologies of the three exposure intensities. As frequency increased the percentage of ears with pathologies decreased.

2. Anatomic Correlates of Noise-Induced Hearing Loss

This study reviewed the state of knowledge concerning the relationship between anatomic evidence of cochlear damage and the parameters of noise exposure that induced hearing loss in laboratory animals. Various anatomic changes induced by noise trauma were reviewed. These changes include damage to sensory cilia and supporting cells, hydrops, pathology of the stria vascularis and external sulcus cells.

For a detailed report see Addendum V.

3. Acoustic Reflex Decay in Chinchillas during a Long-Term Exposure to Noise

Reflex decay to 0.5 kHz octave-band noise at 95 dB SPL was measured in chinchillas during four 2-hour exposure periods separated by 11-minute quiet intervals. Round window electrodes were implanted in six animals. Measures of acoustic reflex decay were inferred from amplitude changes in the cochlear microphonic generated by the octave-band noise. Reflex decay followed essentially the same time course during each exposure period. Vigorous muscular contraction to the signed onset was followed by gradual decay that asymptoted between 30 and 50% of its initial value. The process of decay seemed to be complete some time between 8 and 30 minutes. The results suggest that the middle ear muscles in chinchillas provide some protective function during exposures of fairly long duration.

For a detailed report see Addendum VI.

4. Temporary Threshold Shift following 24-Hour Noise Exposure

Nine men were exposed to 24 hours of continuous noise in a sound field. The noise was an octave band centered at 4 kHz at levels 80 and 85 dB. Hearing thresholds were measured monaurally at 11 test frequencies ranging from 250 to 10000 Hz before, during, and after exposure. Temporary threshold shift (TTS) reached maximum levels at 8 to 12 hours exposure. Maximum TTS occurred at 4 and 6 kHz. Mean asymptotic threshold shifts (ATS) resulting from the 80-dB exposure level were 9.3 dB for 4 kHz and 7.2 dB for 6 kHz. For the 85-dB noise level, these theshold shifts were 17.8 dB and 14.6 dB respectively. The increase in ATS with increase of noise level for these two frequencies could be fitted with a straight line having a slope of 1.6.

For a detailed report see Addendum VII.

5. Asymptotic Threshold Shift in People with Sensorineural Hearing Loss

Twelve men with mild to moderate sensorineural hearing loss in the frequency range of 3-6 kHz were exposed to 24 hours of continuous noise. The noise was an octave band centered at 4 kHz at a level of 85 dB. Hearing thresholds were measured monaurally at 11 test frequencies ranging from 250 to 10,000 Hz prior to exposure and at selected intervals during and after exposure. Temporary threshold shift (TTS) development followed a similar time course to that observed in normal-hearing subjects, asymptotic levels being reached between 8 and 12 hours of noise exposure. Maximum TTS occurred at 4 and 6 kHz. The amount of TTS was less for the subjects with sensorineural hearing loss than for people with normal hearing. However, the sound pressure level required to detect pure tone (shifted thresholds) following noise exposure was greater in the group with hearing loss than was measured in the normal-hearing subjects. Within the limits of this experiment, a sensorineural hearing loss does seen to exert a significant effect on change in hearing sensitivity resulting from noise exposure.

For a detailed report see Addendum VIII.

6. Reflex Threshold Shift in Chinchillas following a Prolonged Exposure to Noise

The acoustic reflex is considered to reduce transmission across the middle ear and thereby protect the inner ear from intense sounds. The dynamic properties of this reflex seem to be a function of the duration of the eliciting stimulus. Assessment of the protective action afforded by middle ear muscle contractions for long-term noise exposures requires the knowledge of how these dynamic properties change under such conditions. Round window electrodes were implanted in eight chinchillas. Changes in the threshold of the acoustic reflex were measured during an 8-hour exposure at 95 dB SPL to an octave-band noise centered at 0.5 kHz. The criterion measure of the acoustic reflex was a change in the amplitude of the cochlear microphonic generated by a 0.5 kHz eliciting tone. Thresholds of the acoustic reflex increased systematically throughout the noise exposure up to approximately 14 dB after 8 hours. The time course of the changes in the threshold of the acoustic reflex was nearly identical to the time course of behaviorally measured changes in the auditory sensitivity as reported by Carder and Miller (1972).

For a detailed report see Addendum IX.

7. Effects of Prolonged Noise Exposure on Chinchillas with Severed Middle Ear Muscles

Using round window-recorded CM as the index of acoustic reflex activity we noted a decay in the strength of middle ear muscle contraction in the chinchilla following an 8-hour exposure to octave-band noise (500 Hz CF, 95 dB SPL). Based on this observation, we concluded that the prolonged exposure reduced the effectiveness of the acoustic reflex in protecting the cochlea. This reduction, however, may have been underestimated if the exposure was also sufficient to reduce cochlear output. The present investigation examined this possibility by comparing the effects of a similar exposure in chinchillas with intact (normal) and severed middle ear muscles.

Following exposure, CM magnitude increased slightly in the normal group. CM decreases were observed in the animals with severed muscles, even when the overall exposure level was reduced to simulate the effects of middle ear muscle contraction. These findings indicate that although the muscles did afford some degree of protection to the cochlea during the exposure, the protective effects of the acoustic reflex may have been reduced even beyond our original observations.

8. Lower Intensity Limits of Noise Which Produces Measurable TTS following 24-Hour Exposure*

An important parameter for assessing the risk for hearing damage from noise exposure is the lowest level of acoustic energy capable of producing significant lasting change in hearing sensitivity. If the

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*Presented by William Melnick in a poster session at the 97th meeting of the Acoustical Society of America, M.I.T., Cambridge, Mass., June 15, 1979.

assumption is valud that noise that does not produce a measurable temporary change in hearing sensitivity (NIPTS) will not produce permanent hearing loss (NIPTS), and if asymptotic threshold shift (ATS) represents the limit of predictable hearing loss that would be expected from a given noise condition, then the measurement of the lowest intensity capable of producing ATS should provide a valid estimate of the upper limits of safe noise levels. The purpose of this study was to determine the level of noise necessary to produce measurable ATS following 24 hours of exposure interrupted only to assess hearing sensitivity.

a. Experimental Conditions

Subjects: 10 adult males with normal hearing for each of 4 noise levels. Normal hearing is defined as threshold hearing levels of 15 dB or less for the audiometric frequencies.

Test Frequencies: 250, 500, 1000, 1500, 2000, 3000, 4000, 6000, and 8000 Hz.

Test Technique: Monaural, self-recording audiometry. Threshold was tracked for 40 seconds at each frequency.

Pre-Exposure Estimate of Hearing Sensitivity: Mean of 10 measures for each frequency taken prior to exposure.

Test Intervals: During exposure; 1, 2, 4, 8, 16, and 24 hours into exposure. Following exposure: 1, 2, 4, 8, and 24 hours.

b. Results

Figure 1 is a diagram indicating the arrangement of the exposure environment. The room was an IAC double-walled sound treated chamber. Hearing testing and exposure took place in the same location.

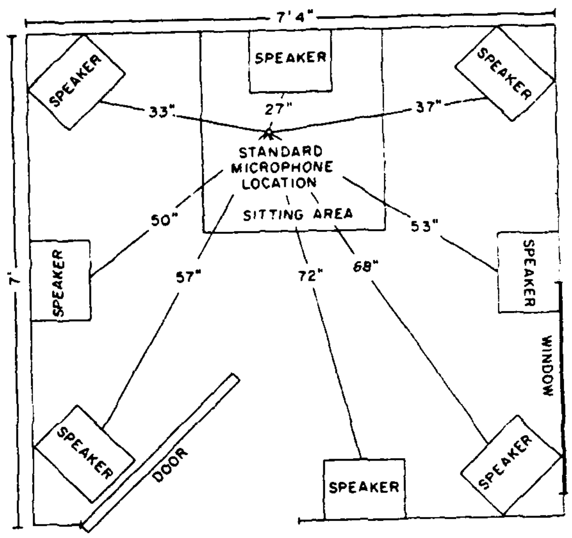
7'4"
7'
SPEAKER
SPEAKER
SPEAKER
33"
27"
37"
STANDARD
MICROPHONE
LOCATION
SITTING AREA
50"
53"
SPEAKER
SPEAKER
57"
72"
68"
WINDOW
SPEAKER
DOOR
SPEAKER
SPEAKER

Fig. 1

The spectrum of the sound field as determined by a 1/3 octave-band analysis is shown in Figure 2. The data points represent the mean for observations made at 27 locations in the sound field.

The mean threshold hearing levels for the 10 test subjects used in each of the exposure conditions are presented in Figure 3. The bars in the figure represent the standard deviation for these measures.

ATS was determined by averaging the temporary threshold shift (TTS) measured at 8, 16, and 24 hours of exposure. The mean ATS as a function of frequency is graphed in Figure 4 for each of the noise levels 76 through 85 dBA. The only statistically significant shifts occurred at L_A of 85 dB for the frequencies 1000, 1500, and 4000 Hz. Despite the lack of statistical significance there does seem to be a systematic increase in ATS as the noise levels increase, particularly at 1.0 and 1.5 kHz.

Figures 5A, B and C show the development and recovery of TTS for the test frequencies 250, 1000, and 4000 Hz. The measurements at 250 Hz show no systematic change with duration and exposure level. The results for 1000 and 4000 Hz show a systematic progression of threshold change with both duration and intensity. The pattern of TTS development in these figures could be interpreted as indicating that ATS was not reached in this 24-hour exposure period.

c. <u>Conclusions</u>

(1) The only statistically significant threshold shifts measured following a 24-hour exposure occurred at 85 dBA and at the frequencies 1000, 1500, and 4000 Hz.

(2) There was a trend for systematic development over the 4 levels of exposure, particularly at 1000 and 1500 Hz.

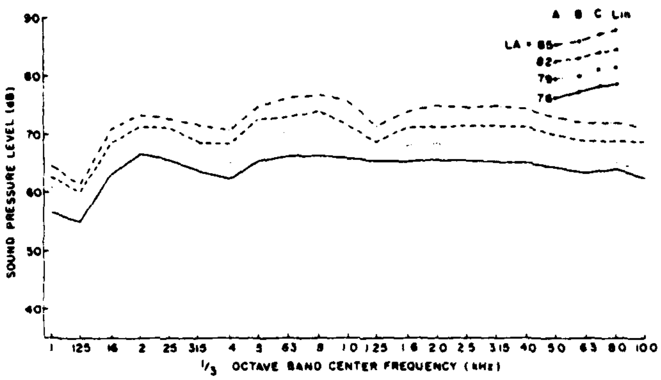

Fig. 2

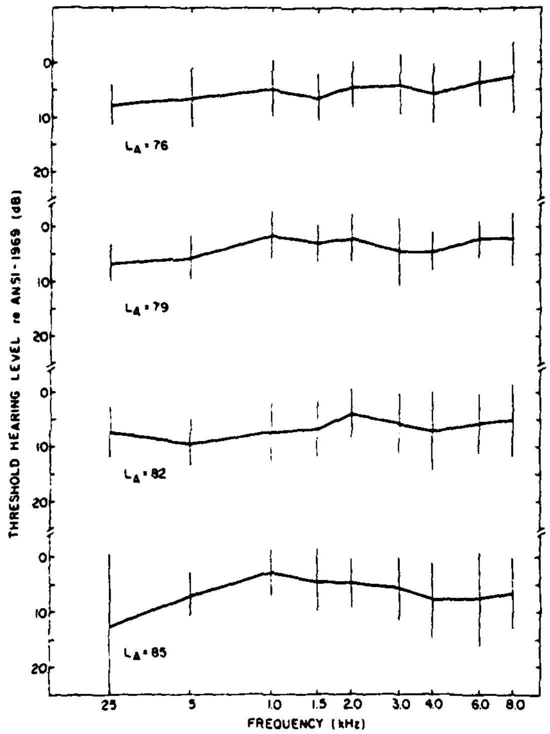

Fig. 3

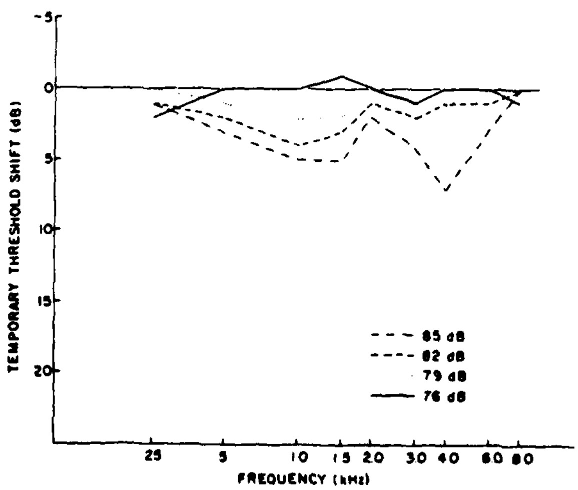

Fig. 4

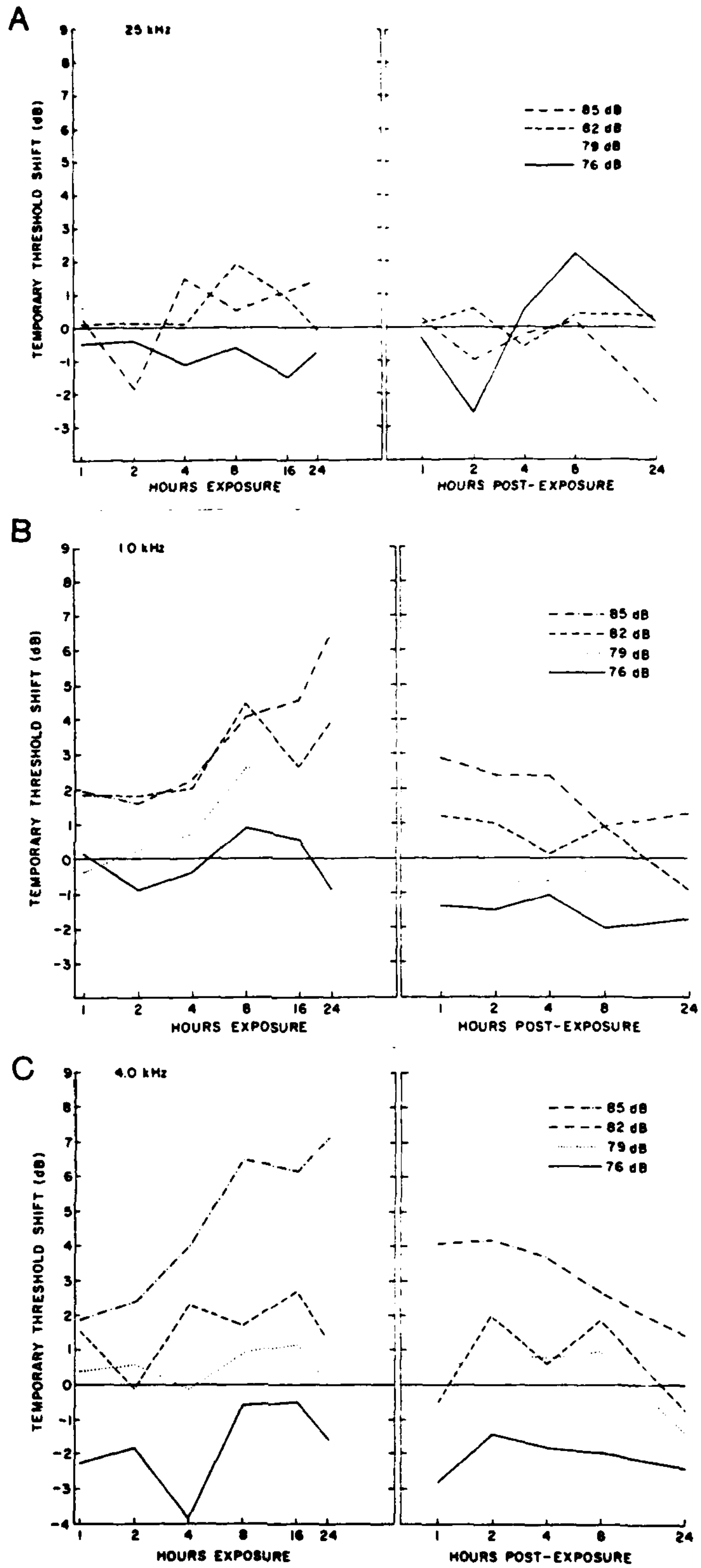
A
B
1.0 kHz
C
4.0 kHz
TEMPORARY THRESHOLD SHIFT (dB)
85 dB
82 dB
79 dB
76 dB
HOURS EXPOSURE
HOURS POST-EXPOSURE

Fig. 5

(3) Asymptotic threshold shifts may not have been reached by 24 hours in this experiment.

(4) These results would support the contention that broadband noise levels of 80 dBA or less could be considered safe.

9. Effects on the Auditory System from in Utero Noise Exposure in Lambs

The purpose of this study was to determine if the auditory systems of lambs would exhibit structural or hearing damage as a result of noise exposure during fetal development.

A total of 18 lambs were used, of which nine were exposed to noise during their fetal development and nine control lambs were exposed only to normal environmental sounds pre- and postnatally. All the lambs had their auditory sensitivity assessed at between 30 and 40 days of age using scalp electrodes to measure brainstem evoked response (BER) to click stimuli. The BER thresholds for all lambs were found to be within the range of normal auditory sensitivity for this procedure.

Following the BER measurements, the lambs were sacrificed and the temporal bones removed, embedded in celloidin, and serially sectioned for evaluation under the light microscope. During the assessment of auditory sensitivity and the evaluation of cochlear morphology, the investigators were not informed about the lambs' noise exposure history.

The morphological evaluation revealed abnormalities in both the control and exposed cochleas. The control ears (Table 1) exhibited cochlear hydrops in 3 of the 18 ears (16%), abnormal or missing supporting cells in the organs of Corti in 4 of 18 ears (22%), and collapsed Reissner's or collapsed or fused tectorial membranes in 4 ears (22%). The exposed ears

Table 1. Cochlear pathologies in control lambs.

LAMB #		Reissner's Membrane Pathology	Tectorial Membrane Pathology	Pillar Cell Pathology	Claudius' Cell Pathology	Hensen's Cell Pathology	Cochlear Hydrops
1	L						
	R						X
4	L						
	R						X
5	L		X	X			
	R	X					
8	L						
	R						
9	L						
	R	X	X				
12	L						
	R						
13	L						
	R						
16	L				X	X	
	R						
18	L						
	R	X			X	X	X

(Table 2) exhibited the same morphological anomalies, but the frequency and severity of the conditions were greater in the exposed groups. Seven of the 18 exposed ears (38%) exhibited cochlear hydrops, 10 ears (55%) showed signs of damage or degeneration in the supportive cells of the organ of Corti, and 6 (33%) had anomalies of Reissner's or the tectorial membrane.

Blood vessels were found on Reissner's membrane and running between Reissner's membrane and the walls of the cochlear scalae and stria vascularis in both the control and experimental animals. Even though the exposed ears exhibited almost twice the number of morphological anomalies found in the control group, we interpreted these data to be histological artifacts since the control ears also displayed these same anomalies. However, the possibility that the neonatal noise exposure may have delayed the development of the organ of Corti could not be ruled out.

C. Publications Resulting from This Study

1. In Print

a. Lim, D.J.: Current review of SEM techniques for inner ear sensory organs. Scanning Electron Microscopy/1977/II, IIT Research Institute, Chicago, Ill., 60616, pp. 401-408.

b. Lim, D.J.: Ultra anatomy of sensory end-organs in the labyrinth and their functional implications. Proc. of the Shambaugh Fifth International Workshop on Middle Ear Microscopy and Fluctuant Hearing Loss, G.E. Shambaugh, Jr. & J.J. Shea (eds.). Huntsville, Ala., Strode Publishers, 1977, pp. 16-27.

Table 2. Cochlear pathologies in lambs exposed to noise *in utero*.

LAMB #		Reissner's Membrane Pathology	Tectorial Membrane Pathology	Pillar Cell Pathology	Claudius Cell Pathology	Hensen Cell Pathology	Cochlear Hydrops
2	L	X		X	X	X	
	R	X	X	X			
3	L	X	X		X	X	
	R						
6	L	X	X	X	X		X
	R	X	X	X	X	X	
7	L						
	R						
10	L	X	X		X	X	
	R						
11	L				X	X	X
	R					X	
14	L						
	R				X	X	X
15	L						X
	R						X
17	L						X
	R				X	X	X

c. Viall, J., Melnick, W.: Asymptotic threshold shift in people with sensorineural hearing loss. Trans. Amer. Acad. Ophthalmol. Otolaryngol., 84:ORL-459-ORL-464, 1977.

d. Lim, D.J.: Fine morphology of the tectorial membrane: Fresh and developmental. Inner Ear Biology, M. Portmann & J.-M. Aran (eds.). Paris, INSERM, 68:47-60, 1977.

e. Melnick, W.: Temporary threshold shift following 24-hour noise exposure. Ann. Otol. Rhinol. Laryngol., 86:821-826, 1977.

f. Gerhardt, K.J., Melnick, W., Ferraro, J.A.: Reflex threshold shift in chinchillas following a prolonged exposure to noise. J. Speech Hear. Res., 22:63-72, 1979.

g. Lim, D.J., Dunn, D.E.: Anatomic correlates of noise-induced hearing loss. Otol. Clin. N. Amer., 12:493-513, 1979.

h. Gerhardt, K.J., Melnick, W., Ferraro, J.A.: Acoustic reflex decay in chinchillas during a long-term exposure to noise. Ear & Hearing, 1:33-37, 1980.

Lim, D.J.: Cochlear anatomy related to cochlear micromechanics: A review. J. Acoust. Soc. Amer., 67:1686-1695, 1980.

2. In Press

a. Lim, D.J., Dunn, D.E., Johnson, D.L., Moore, T.J.: Trauma of the ear from infrasound. Accepted for publication in Acta Otolaryngol.

b. Ferraro, J.A., Melnick, W., Gerhardt, K.: Effects of prolonged noise exposure on chinchillas with severed middle ear muscles. Submitted to Amer. J. Otolaryngol.

3. In Preparation

Lim, D.J., Dunn, D.E., Ferraro, J.A., Moore, T.J.: Effects on auditory system from in utero noise exposure in lambs.

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